

METHODS OF ISOLATION AND CULTURING OF *M. ulei*

Microcyclus ulei

- The fungus has been successfully cultured on artificial media;
- On solid media, the fungus form dark stroma which is raised above the surface of the medium. Certain isolates especially of Race 2 grows on the surface and underneath the surface of the media;
- The growth of the stroma is very slow and is not suitable for quick diagnostic purposes;
- Cultures of *M. ulei* produces conidia but not ascospores;

M. ulei cultures on solid medium



FUNGAL ISOLATION USING SECTION OF INFECTED LEAF

- The fungus can be isolated by culturing infected leaves or by direct transfer of conidia;
- Water agar is used as the isolation medium;
- Isolation by culturing lesions:
 - The leaves should be harvested on a dry day to reduce contaminant and fresh lesions are selected;
 - Surface sterilized a small section of leaf with lesion in mercuric chloride for 30 sec and transferring to medium. The leaf sections can also be sterilized with sodium hypochlorite;
 - Some scientist prefer to cut the leaf section across the lesion and not around a lesion for better success;
 - Transfer the leaf sections onto growth medium.

FUNGAL ISOLATION USING CONIDIA

- Carefully touch the top surface of the lesion with moist tip of an isolating needle and placing the conidia onto the surface of agar medium contained in test tubes;
- A small piece of the isolation medium (2 x 2 mm) is placed onto the tip of an isolation needle. Carefully touch the surface of lesions with the agar block and later placing the agar block onto the medium; Carefully touch the surface of the lesion with a small piece of agar.

FUNGAL ISOLATION USING CONIDIA (cont.)

- The conidia are deposited onto the surface of water agar by tapping a leaf bearing lesions. With the aid of a stereomicroscope, a piece of the agar with the spores is then transferred onto growth medium. This method is most suitable to obtain a single spore culture.
- Ascospores can also be used for isolation. Section of leaves with perithecia is stuck to the inner surface of a Petri dish cover. The perithecia are moistened with cold water. The cover is then placed on top of Petri plate containing water agar. Under a stereo microscope, the spores are located and the agar is cut and transferred onto the growth medium.
- Antibiotics such as chloroamphenicol at 50 mg/l can be included in the medium.

GROWTH MEDIA (1)

- Solid and liquid media had been developed;
- Various compositions of carbohydrates, amino acids, vitamins and leaf extracts were tried;
- Different media are used for fungal isolation, maintenance and production of conidia;
- Potato sucrose agar (PSA) medium containing 2.5 or 5% sucrose is commonly used to culture *M. ulei*;
- A modified PSA containing multivitamins and minerals was indicated to improve growth;

GROWTH MEDIA (2)

- Various liquid media had been tried. Satisfactory growth occurs on liquid potato sucrose medium or PSA with 0.5% agar.
- Generally, rich media support growth of stroma and the lower sucrose content is better for sporulation.
- In order to increase spore production certain supplements (dog food, coconut water) are added.

GROWTH MEDIA (3)

- For storage, sporulating colonies of the fungus growing on agar slants are submerged with sterile mineral oil. Rejuvenation is often slow;
- For short duration storage, the fungus is grown on egg yolk medium;
- The conidia can also be preserved for several months in infected leaves kept dry in a refrigerator or a dessicator;

INOCULUM PRODUCTION IN CULTURE

- Production of spores in vitro is low especially in rich media;
- PSA with 2.5% sucrose supports production of conidia. PSA amended with peptone or coconut water improve spore production;
- Conidial production is improved by exposing the culture to ultra violet light for 45 min/day for 14 days.

INOCULUM PRODUCTION IN CULTURE (2)

- Alternating exposure to light and dark periods improve spore production:
 - Grow culture in the dark but expose to fluorescent light for 60-90 min/day for 14 days;
 - Grow culture in the dark for 12 days and then expose to fluorescent light for one h per day for the next two days;
 - Expose to 1 hr fluorescent light, 3 h of darkness, 1 h light, 3 h dark, 1 h light and 15 h darkness successively.

FUNGICIDE SCREENING METHODS

- Laboratory methods:
 - Germination of conidia in fungicide solutions or fungicide treated cellophane film disks;
 - Infection of fungicide treated detached leaflets or leaf disks.
- Nursery methods
 - Spraying fungicide onto young leaves.

FUNGAL INOCULATION

- Spores from cultures are harvested by adding water, brushing the surface of culture gently and filtering the suspension using cheese cloth;
- The spore inoculum (2×10^5 spores/ml) is sprayed onto young leaves (copper brown stage). The leaves should be kept under high humidity by keeping in a growth chamber or bagged in plastic bags overnight;
- In the laboratory, leaf disks suspended on water or detached leaf kept in moist containers can be inoculated to verify SALB.